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EDITORIAL COMMENT

Imaging Inflammation in Atherosclerosis

Another Step Forward*

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Atherosclerosis is an immuno-inflammatory disease of the arterial wall involving multiple intertwined biological processes. These processes include nonlaminar flow that creates a pro-inflammatory endothelial phenotype and helps localize atherosclerotic lesions preferentially to regions of disturbed flow; atherogenic lipoprotein entry into the arterial wall leading to their retention and oxidation; inflammatory gene induction with endothelial activation leading to recruitment of mononuclear cells followed by their retention and activation; conversion of monocytes into macrophages and foam cells after ingestion of lipids; smooth muscle cell migration and proliferation; extracellular matrix

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remodeling; cell death; and angiogenesis (1,2). Atherosclerosis takes its greatest toll by triggering thrombosis usually superimposed on a ruptured fibrous cap; however, as many as 30% of thrombi superimposed on plaques might occur in absence of plaque rupture with only superficial endothelial erosions (1–3). Nearly one-half of all potentially lethal acute thrombotic manifestations of atherosclerosis, such as acute myocardial infarction or sudden cardiac death, occur in individuals without prior warning symptoms or knowledge of disease. Although atherosclerosis is a multifocal disease, certain types of plaques, so-called high-risk plaques or vulnerable plaques, are more likely to be associated with thrombosis (1–3). Rupture-prone plaques are characterized by a large lipid-rich core, thinned out collagen and smooth muscle cell depleted fibrous cap, outward remodeling, increased plaque neo-angiogenesis, and increased inflammatory cell infiltration in the fibrous cap and adventitia (2,3). Noninvasive detection of subclinical atherosclerosis in various arterial beds is now possible with ultrasound, computed tomography (CT), and cardiac magnetic resonance imaging; however, identification of plaques that are at high risk for triggering thrombosis in the near-term remains a challenge. Inflammation in atherosclerosis is linked to several biological processes, such as foam cell formation, extracellular matrix degradation and arterial wall remodeling, cell death, plaque neoangiogenesis and intraplaque hemorrhage, plaque progression, lipid core expansion, plaque rupture, and thrombosis (1–3). Noninvasive imaging of molecules and pathways or biological processes linked to inflammation holds promise for identification of high-risk plaques and monitoring the effects of therapeutic interventions (4–7). A number of modalities to image inflammation in the arterial wall are in various stages of preclinical and clinical development (4–6). Positron emission tomography (PET) imaging with ^{18}F-labeled deoxyglucose (^{18}FDG) as a metabolic tracer is one of these techniques (4,5). The ^{18}FDG is widely used clinically for cancer imaging, and recent studies have shown that it also accumulates in the atherosclerotic lesion, presumably indicating enhanced glucose uptake by metabolically active macrophages and possibly other cells (7–9). Despite promising initial clinical results with ^{18}FDG PET imaging in detecting inflammation in human carotid plaques, the possibility that metabolically active cells other than inflammatory cells could also take up ^{18}FDG could reduce the specificity of this approach. Furthermore, the increased background glucose uptake in metabolically active

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myocardium makes imaging of inflammation in human coronary plaques more challenging.

In this issue of *iJACC*, Nahrendorf et al. (10) present their pre-clinical data on imaging vascular cell adhesion molecule (VCAM)-1 by hybrid imaging involving PET as a molecular imaging tool and CT as an anatomical imaging tool in murine atherosclerosis. Recruitment of mononuclear inflammatory cells into the arterial wall is initiated by an interaction between a key adhesion molecule of the immunoglobulin superfamily—VCAM-1—expressed on the activated endothelium and its ligand, very late antigen (VLA)-4 expressed on the leukocyte surface (11). The expression of VCAM-1 is increased at lesion-prone sites and in atherosclerotic plaques, where it is expressed by endothelial as well as smooth muscle cells and macrophages (12). In an attempt to image VCAM-1 expression in the atherosclerotic lesions, the authors first identified a linear synthetic peptide VHPKQHR with sequence homology to VLA-4, with in vivo phage display with 3 rounds of positive iterative selection from aortas of apolipoprotein E (ApoE)$^{-/-}$ mice (10). The increased affinity of VHPKQHR to VCAM-1 was attributed to the multivalent tetrameric nature of the peptide (4V), and its binding to VCAM-1 was shown to be highly specific (10). The 4V was then labeled with ^{18}F (^{18}F-4V) for PET imaging. In addition, the authors also used a fluorescent version of the ^{18}F-4V to allow for rapid identification of the peptide in tissues. Imaging was performed in 3 different settings where VCAM-1 is upregulated: the setting of aortic atherosclerosis in old hypercholesterolemic ApoE$^{-/-}$ mice fed a high fat diet, in a murine model of acute myocardial infarction, and in a murine model of cardiac transplant rejection (10). For atherosclerosis, PET imaging was used on excised murine aortas as well as in intact animals 1 to 2 h after injection of ^{18}F-4V to localize in vivo VCAM-1 expression along with high-resolution contrast enhanced CT to provide anatomic reference to the aortic root. Anatomic location of VCAM-1 expression was verified by immunohistological assessment and with fluorescence microscopy after injection of fluorescence labeled ^{18}F-4V. Further validation of in vivo VCAM-1 imaging results with PET was achieved with comparison with quantitative polymerase chain reaction data for VCAM-1 and macrophage marker CD68 expression in tissue. The authors show that ^{18}F-4V accumulates in atherosclerotic plaques, mostly in the endothelium overlying aortic plaques, and is detected by in vivo and ex vivo PET/CT imaging, and the tracer accumulation correlates reasonably well with polymerase chain reaction evidence for VCAM-1 ($R = 0.79$) and CD68 ($R = 0.5$) expression (10). Furthermore, pre-injection of a specific anti–VCAM-1 monoclonal antibody into the mice markedly reduced ^{18}F-4V uptakes, indicating high level of VCAM-1 specificity of the tracer. The authors also show increased uptake of ^{18}F-4V in myocardium after myocardial infarction and during transplant rejection.

This is an important advance in molecular imaging of vascular inflammation that brings us a step closer to realizing this possibility in humans. The authors provide compelling data to support a reasonably good sensitivity and specificity of their PET tracer and imaging techniques for identifying VCAM-1 as a marker of vascular inflammation. The biodistribution kinetics with a large ratio of uptake in atherosclerotic versus nonatherosclerotic aorta as well as a short circulating half life make the tracer quite attractive for potential human imaging. The authors also show that Atorvastatin treatment in the mouse reduced ^{18}F-4V uptake consistent with its known anti-inflammatory effects, thus suggesting the possibility that this imaging method might have a dynamic range to allow assessment of serial changes and effects of therapeutic interventions on plaque inflammation. That would be yet another step in the right direction.

However, several caveats should serve as a reminder of challenges ahead in translating this work to the study of human atherosclerosis. First, we must acknowledge that the authors did not directly image inflammation but imaged a key molecule that is important in leukocyte-endothelial adhesion, which is an early step in arterial wall leukocyte recruitment. Thus, it is plausible that plaque inflammation could be altered at steps downstream from the initial step of adhesion by interventions that affect leukocyte chemotaxis, activation, survival, or egress from the lesion, which could modulate inflammation in the plaque without a change in VCAM-1 expression. Dansky et al. (13) have shown that, in a murine model, elevated Apo A-I and high-density lipoprotein cholesterol levels inhibit foam cell formation at a stage after subendothelial lipid retention, endothelial VCAM-1 induction, and monocyte adherence. Similarly, an athero-regressive environment was associated with egress of macrophages from the atherosclerotic lesion without affecting the entry of monocytes, leading to a net decrease in plaque inflammation; VCAM-1 imaging in this setting might be unable to detect this phenomenon (14). Second, leukocyte recruitment into the atherosclerotic lesion might also occur through plaque and adventitial neovasculature, and it is unclear how the current methodology would work in that

setting (15). These caveats are reinforced by the author's data, which show that the ^{18}F-4V uptake in the aortic lesions correlated better with VCAM-1 gene expression (R = 0.79) than with macrophage marker CD68 gene expression (R = 0.5). It would have been interesting to know the correlation between the extent of plaque macrophage immunoreactivity (a more direct index of plaque inflammation) and the magnitude of ^{18}F-4V uptake; unfortunately, these data are not provided. Another potential limitation of the imaging modality used by the authors is the emerging evidence of heterogeneity of monocytes and macrophages in mice and humans, where different subsets might have pro-inflammatory or possibly anti-inflammatory pro-healing effects that could not be distinguished on the basis of VCAM-1 imaging (16). Although the approach used by authors seems to have worked reasonably well in the murine aortic root atherosclerosis model, it remains to be seen whether it would be equally good for imaging other relevant vascular sites, such as the coronary arteries and the carotid arteries. Finally, although in humans inflamed lipid-rich plaques are prone to rupture and thrombosis, nearly one-third of coronary thrombi evolve from plaques that do not have a large lipid core or significant inflammation but have superficial endothelial erosions; it is unlikely that this subset of high-risk plaques would be definitively identified with VCAM-1 imaging (3).

Notwithstanding these potential limitations, the molecular imaging approach developed by the authors is definitely a step forward in our quest for imaging at least an important subset of high-risk plaques. It will also be of great interest to learn how the approach used by Nahrendorf et al. (10) will compare with alternative imaging modalities already under clinical investigation for structural and functional imaging of human atherosclerosis, such as aortic and carotid magnetic resonance; contrast enhanced CT coronary angiography; and ^{18}FDG PET imaging of inflammation in aorta, carotid arteries, and coronary arteries (in combination with CT) (4–9,16,17).

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